Geomagnetic Navigation in Monarchs and Black Swallowtails[1]

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Abstract. Monarch butterflies (*Danaus plexippus*) in North America migrate to and from Mexico. Black swallowtails (*Papilio polyxenes*) are non-migratory and travel locally. Two hypotheses have been suggested concerning the navigation of monarchs: that the monarchs use an internal sun compass, or that they use a geomagnetic compass. The data collected by this research show that both species have the ability to use geomagnetic navigation and that monarchs do, in fact, use geomagnetic navigation. Neutron activation analysis was used to assay iron concentrations by species, body parts, and sex. It was shown that the head had the highest iron concentrations of the body parts, with monarch females being higher than monarch males. The gender pattern was reversed in the black swallowtails. A strong magnet and insect pavilion was used in darkness and sunlight in different orientations to test the hypothesis that monarchs have a geomagnetic sensory system and use geomagnetic navigation. Monarchs were affected by the magnet in both sunlight and dark, while black swallowtails did not show conclusively that they use geomagnetic navigation. These findings may have parallels in other migratory and non-migratory species of animals.

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INTRODUCTION

Previous studies have speculated that monarchs use the sun in a continual clockwise orientation throughout the year (Brower 1996; Reppert and others 2004) or a circadian clock (Froy and others 2003) for navigation. Other work suggests that monarchs navigate by the earth's magnetic field instead of the sun (Etheredge and others 1999). Geomagnetic navigation has been found in a variety of animals such as salmon, tuna, and some birds. Non-migrators, such as some bacteria, amphipod sandhoppers, newts, and the American alligator (Milius 2004), use it as well. The European robin was recently added to the migratory list (Ritz and others 2004). It has also been shown in honeybees (Frier and others 1996; Kirschvink 1981), mosquitoes (Strickman and others 2000), blind mole rats (Kimchi and Terkel 2001), spiny lobsters (Boles and Lohmann 2003), and green sea turtles (Lohmann and others 2004).

It was hypothesized that if the head of monarchs and black swallowtails contained higher iron concentrations than the thorax, abdomen, and wings, they would have the ability to use geomagnetic navigation. This project investigated the ferromagnetic content of monarchs and black swallowtails by Neutron Activation Analysis. Neutron Activation Analysis was used because of its accuracy in measuring low elemental concentrations in samples with low total masses. The suggestion that monarchs can perceive magnetism leads to the conclusion that there must be a higher concentration of iron in the head, which would indicate a geomagnetic sensory system.

The iron concentration of milkweed was also of interest as it is the primary source of food for the caterpillar stage of the monarch. Analysis of washed and unwashed milkweed leaves was conducted to test for environmental pollution.

MATERIALS AND METHODS

Monarchs were collected from Kansas during fall migration, from Mexico while overwintering, and from Ohio during the summer and fall. Black swallowtails were collected from Ohio during the fall. These samples were dried, either ground whole or separated into heads, thoraxes, abdomens, and wings, and placed in snap-top polyethylene vials, volume 0.15 ml. Empty vials were weighed to the nearest hundredth of a milligram and then weighed when full, and the mass of the sample was calculated. Analysis of washed and unwashed milkweed leaves was conducted to test for environmental pollution. A nuclear reactor was used to irradiate the samples for three hours at 350 kW of power. The samples were then removed from the reactor and analyzed for gamma emissions. Calibration of the instrument used standards with known elemental contents, which were counted, plotted on a graph, and fit with a linear regression model to determine the relationship between counts and iron concentrations (in ppm). The calibration line was used to estimate the iron concentration in the butterfly samples.

Statistical analyses summarize the iron concentration data and determine differences in iron concentrations between sexes, body parts, and species. Because the distribution of iron concentrations was positively skewed, natural logarithms of iron concentrations were used in the analysis. In addition to geometric means, a three-factor analysis of variance (ANOVA) was used.

To test if the butterflies actually used geomagnetic navigation, research was conducted to determine if it was possible to change the butterflies' orientation. A very strong horseshoe magnet from a nuclear accelerator was used to overcome the earth's magnetic field in the area around an insect pavilion. The pavilion was constructed

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of nylon mesh in the shape of a tube 6.0 ft long and 2.0 ft in diameter. Groups of 9 monarchs and black swallowtails were placed in the insect pavilion under the following conditions: in total darkness in the pavilion, with the magnet suspended at one end of the pavilion, which was rotated every 24 hours in north – south orientation with and without the magnet at south end; outside in natural sunlight, with a north – south orientation, with magnet at south end; outside in natural sunlight, with a north – south orientation without magnet.

The results were recorded at the same time every day. The experiments were repeated three times.

Results of Iron Concentration Analysis

No significant trends were found between the washed and unwashed samples of milkweed indicating the lack of environmental pollution. It was noted however that the analysis of the milkweed leaves showed a strong trend of increasing iron concentration the closer the leaves were to the base of the plant. The top leaf only had 1/3 of the iron compared to the bottom leaf. This was expected because as the plant matures more minerals are accumulated in the leaf tissue (Mills and Jones 1996). Laboratory monarchs raised in sterile conditions without any outside pollution actually had elevated iron concentrations relative to natural monarchs due to the diet fed to the caterpillars. Table 1 provides an overview of the results of the iron analysis for the butterflies. Average iron concentrations are presented for each study, for each sex within study, and for each body part (and over all body parts).

The migrating monarchs from Kansas, all of which were male, were dissected into four parts—heads, thoraxes, abdomens, and wings—which were analyzed separately. The overall average iron concentration found in the Kansas monarchs was 98.48 ppm. When the body parts were analyzed separately, the iron concentrations found were 105.5 ppm in the heads, 105 ppm in the thoraxes, 88.66 ppm in the abdomens, and 97.66 ppm in the wings. The monarchs found in Mexico were also all male; they were analyzed separately as whole butterflies. Their overall average iron concentration was 88.5 ppm.

The Ohio summer monarchs, which were all caught on 10 July 2002, were separated by sex and analyzed by body parts. The average iron concentration found in Ohio summer monarchs was 67.4 ppm for the females and 53.14 ppm for the males. When the body parts were analyzed separately, the females' and males' iron concentrations found were 224 and 114 ppm in the heads, 73 and 42 ppm in the thoraxes, 25 and 52 ppm in the abdomens, and 81 and 63 ppm in the wings, respectively. The Ohio fall monarchs, which were caught on 25 September 2002, were separated by sex and analyzed by body parts. The average iron concentration found in the Ohio fall monarchs was 58.4 ppm for the females and 46.2 ppm for the males. When the body parts were analyzed separately, the females' and males' iron concentrations found were 252 and 66.5 ppm in the heads, 42 and 52.5 ppm in the thoraxes, 72 and 37 ppm in the abdomens, and 44 and 43.5 ppm in the wings, respectively. The black swallowtails were separated by sex, with the females and males showing average iron concentrations of 69.2 and 182.9 ppm, respectively. When the body parts were analyzed separately, the females' and males' iron concentrations were 281 and 401 ppm in the heads, 51 and 145 ppm in the thoraxes, 30 and 64 ppm in the abdomens, and 108 and 330 ppm in the wings, respectively (Table 1).

TABLE 1

Species, location, gender, and body part ppm values.

Species	Sex	Mean Iron Concentration (ppm)					
		Whole	Head	Thorax	Abdomen	Wings	# of Samples
Kansas Monarchs	Male	98.48	105.5	105	88.66	97.66	3 sets of 3
Mexico Monarchs	Male	88.5					6
Summer Monarchs	Female	67.4	224	73	25	81	1 set of 3
	Male	53.14	114	42	52	63	1 set of 3
Fall Monarchs	Female	58.4	252	42	72	44	1 set of 2
	Male	46.2	66.5	52.5	37	43.5	2 sets of 2
Lab Monarchs	Female	312					3
	Male	273.5					3
Black Swallowtails	Female	69.2	281	51	30	108	1 set of 3
	Male	182.9	401	145	64	330	1 set of 3

The measure of central tendency appropriate with logarithmically-transformed iron concentrations is the geometric mean, with the geometric standard deviation representing the variability in the data. These values have been calculated for the various species, sexes, and body parts and are displayed in Table 2.

TABLE 2

Measures of central tendencies and variability by species, sex, and body part.

Species	Sex	Body Part	Number of Samples	Geometric Mean	Geometric Standard Deviation
Monarch	Female	Head	2	237.588	1.08685
		Thorax	2	55.371	1.47828
		Abdomen	2	42.426	2.11271
		Wings	2	59.699	1.53959
	Male	Head	5	89.001	1.31576
		Thorax	6	70.325	1.58666
		Abdomen	6	59.780	1.58402
		Wings	6	67.971	1.61609
Swallowtail	Female	Head	1	281.000	NA
		Thorax	1	51.000	NA
		Abdomen	1	30.000	NA
		Wings	1	108.000	NA
	Male	Head	1	401.000	NA
		Thorax	1	145.000	NA
		Abdomen	1	64.000	NA
		Wings	1	330.000	NA

Table 3 contains the results of the three-way ANOVA, including the sources of variation, the degrees of freedom, the sum of squares, the mean square, the F statistic and the p value. The results of the ANOVA indicate that all of the model factors are statistically significant at the 0.10 significance level, with the exception of the sex main effect. However, there are statistically significant interactions between sex and the other two factors. To illustrate where there are differences in iron concentrations between sexes, species, and body parts, three interaction plots have been included as Figures 1 through 3. Figure 1, which shows interactions between body parts and sex, shows that: 1) on average, the butterfly heads have higher iron concentrations than the other three body parts; 2) on average, there is no difference between male and female butterflies; and 3) males have lower levels of iron in their heads than females, while the levels of iron in the other three body parts for males is slightly higher than in females.

Figure 2, which shows the interaction of body parts and species, shows that: 1) on average, swallowtails have higher iron concentrations than monarchs; 2) on average, there are higher iron concentrations in butterfly heads than in the other three body parts; and 3) swallowtails have higher iron concentrations in their heads and wings than monarchs, while iron levels in thoraxes and abdomens are similar in the two species.

TABLE 3

Results table for three-factor ANOVA.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Statistic	P Value
Part	3	5.42709146	1.80903049	10.12	0.0001
Sex	1	0.03225970	0.03225970	0.18	0.6744
Sex*Part	3	2.00615862	0.66871954	3.74	0.0233
Species	1	2.02763493	2.02763493	11.34	0.0024
Part*Species	3	1.45487315	0.48495772	2.71	0.0654
Sex*Species	1	1.17105192	1.17105192	6.55	0.0166
Model	12	12.11906978	1.00992248	5.65	0.0001
Error	26	4.64703446	0.17873209		
Total	38	16.76610423			

Figure 3, which shows the interaction of species and gender, shows that: 1) on average, swallowtails have higher iron concentrations than monarchs; 2) on average, there is no difference between male and female butterflies; and 3) male swallowtails have higher iron concentrations than male monarchs, while there is no difference in iron levels between female swallowtails and monarchs.

The interaction plots, together with the raw ppm data, show that: 1) on average, black swallowtails as a species have higher iron concentrations than monarchs with the black swallowtails males exhibiting the majority of the difference; 2) on average, female monarchs have higher iron concentrations in their heads than male monarchs; and 3) on average, male black swallowtails have higher iron concentrations in their heads than female black swallowtails.

RESULTS

Figures 4 and 5 show the results of the magnet experiment. In the total darkness experiment with the insect pavilion, 100% of male and female monarchs were in the correct southerly portion of the insect pavilion when the insect pavilion was in a north – south orientation without the magnet. When the insect pavilion had the magnet placed at south end, 67% of the female monarchs and 33% of the male monarchs were in the end of the pavilion away from the magnet. In the sunlight experiment with the insect pavilion in a north – south orientation with the magnet at the south end, 50% of the female monarchs and 25% of the male monarchs were in the north end away from the magnet. Without the magnet, 100% of the female monarchs and

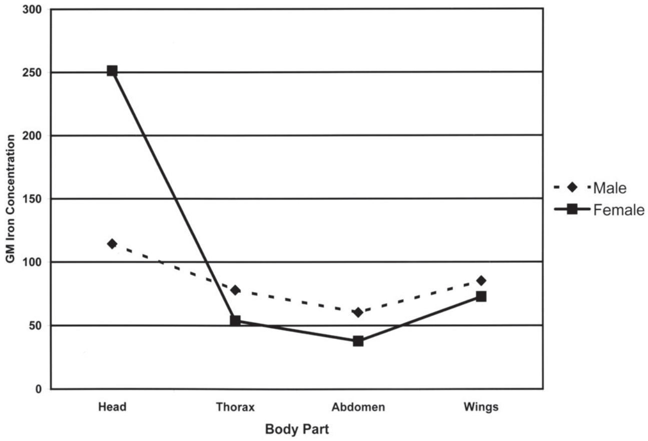

FIGURE 1. Interaction plot for sex and body part.

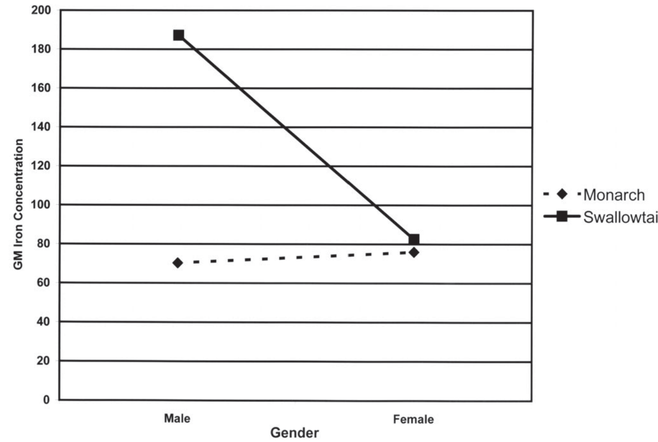

FIGURE 3. Interaction plot for sex and species.

50% of the male monarchs were in the south end of the pavilion. The black swallowtails did not exhibit a pattern and appeared to be evenly located throughout each of the experiments.

DISCUSSION

Data analysis showed that both monarchs and black swallowtails had higher iron concentrations in their heads than the other body parts. The difference between the two was that the sex was reversed between the two species in regards to which had higher iron concentration in their head. This is consistent with the hypothesis that monarchs and black swallowtails have the ability to use geomagnetic navigation. Female monarchs always had higher iron concentration than males, regardless of where or when the monarchs were collected across the United States. This leads one to consider the possibility that the females are better navigators and could possibly increase the chance of a female monarch reaching its destination and reproducing, thus helping to perpetuate the species. Ferromagnetic analysis to conclude that geomagnetic navigation is possible was also used in the studies of honeybees and mosquitoes, previously cited, although different methods were applied.

After determining monarchs and black swallowtails have the ability to use geomagnetic navigation by Neutron Activation Analysis, the second part of the research tested these two species to determine if they actually used geomagnetic navigation. The paper on the European robin employed a similar technique of using a magnet to change the bird's orientation. In our research the insect pavilion and artificial magnetic field provided the answer for the monarchs. The results show that both in sunlight and darkness, monarchs can be tricked into traveling the wrong direction by use of a strong magnet. Thus, it appears that geomagnetic navigation is used by monarchs both in sunlight or when sunlight conditions are not favorable. However, fewer monarchs were tricked in sunlight than in darkness, indicating that sunlight helps overcome the magnet, though not completely. Black swallowtails do not migrate and thus do not have a long-range destination like monarchs, which is why they were evenly located in the magnet experiments in the research. Therefore, it cannot be concluded that black swallowtails use geomagnetic navigation based on this experimentation, although they do have the ability to do so based on their iron concentration. However, black swallowtails are very adept in locating small and isolated habitat patches where food plants grow. Since a need for the species exists to navigate locally

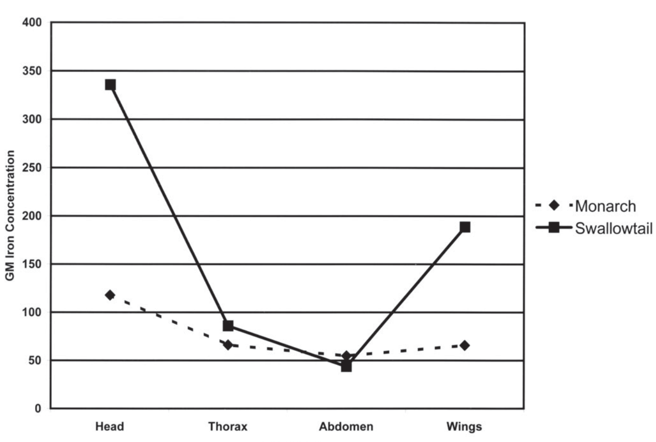

FIGURE 2. Interaction plot for species and body part.

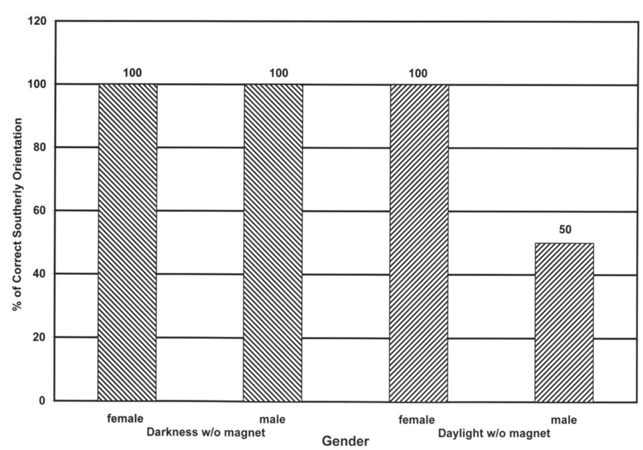

FIGURE 4. Monarch orientation with Earth's magnetic field.

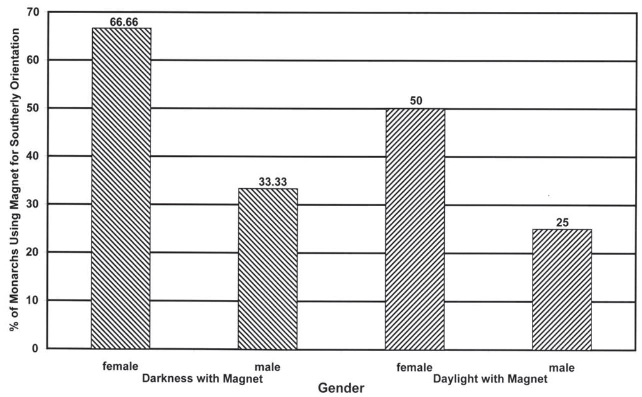

FIGURE 5. Monarch orientation with magnet.

and return to those same feeding areas, geomagnetic navigation cannot be ruled out, as it may be the mechanism used.

This also leads one to speculate that perhaps other migratory species may exhibit similar patterns in the female sex, where it is more important that more females than males to migrate. One can also speculate that other butterfly species may use geomagnetic navigation as it was shown that black swallowtails have the ability to do so.

In conclusion, butterflies have very erratic flight patterns and unless they migrate, it is difficult to test how they navigate. Knowledge acquired by this research may be applicable to other migratory and non-migratory species with greater economic impact. However, for sheer drama and beauty, the Monarch's magnificent migration is hard to match.

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